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Research Article

Morphological and phylogenetic analyses reveal four novel species of *Distoseptispora* (Distoseptisporaceae, Distoseptisporales) from southern China

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Abstract

Saprobic hyphomycetes with high density and species diversity were observed on plant debris. During our mycological surveys in southern China, numerous strains were isolated from dead branches of unidentified plants in the forest of Jiangxi and Fujian provinces, China. Phylogenetic analyses of ITS, LSU, *RPB2*, and *TEF1* sequence data using maximum-likelihood (ML) and Bayesian inference (BI) methods revealed the systematic placement of several *Sporidesmium*-like species within *Distoseptispora*. Based on morphological characteristics and molecular evidence, four new species of *Distoseptispora*, namely *D. fujianensis*, *D. ganzhouensis*, *D. nanpingensis* and *D. subtropica*, are introduced, and two known species, *D. clematidis* and *D. yunjushanensis*, are reported. This study enhances our knowledge of the species diversity of *Distoseptispora* in southern China.

Key words: Asexual fungi, molecular phylogeny, new species, Sordariomycetes, taxonomy

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Introduction

The genus *Distoseptispora* K.D. Hyde, McKenzie & Maharachch., typified by *D. fluminicola* McKenzie, Hong Y. Su, Z.L. Luo & K.D. Hyde, was introduced by Su et al. (2016) based on molecular data and is mainly characterized by acrogenous, solitary, distoseptate conidia seceding schizolytically from monoblastic, integrated, terminal, determinate conidiogenous cells on macronematous, unbranched, septate conidiophores. Subsequently, the generic concept has been expanded by adding species that produce euseptate or muriform conidia (Xia et al. 2017; Yang et al. 2018) and polyblastic conidiogenous cells (Hyde et al. 2019). To date, 84 epithets of *Distoseptispora* are listed in Index Fungorum (Index Fungorum 2024), with all species identified based on morphological and phylogenetic analyses. However, *D. submersa* Z.L. Luo, K.D. Hyde & H.Y. Su (Luo et al. 2018), a phylogenetically close species, showed larger conidiophores (55–73 × 7–9 µm vs. up to 40 × 4–6 µm) than the holotype of *D. tectonae* Doilom & K.D. Hyde (Hyde et al. 2016). Another collection of *D. tectonae* (MFLU 20–0262) also showed varied, larger conidiophore dimensions (34–95 × 5–8 µm)

(Sun et al. 2020). As a result, Dong et al. (2021) synonymized *D. submersa* with *D. tectonae* based on high homology in nucleotide sequences and morphological similarity. Thus, *Distoseptispora* currently includes 83 valid taxa.

Distoseptispora is one of the *Sporidesmium*-like genera found in freshwater and terrestrial habitats. In recent years, the number of *Distoseptispora* species has steadily increased, primarily based on morphological and phylogenetic analyses. Recent studies have indicated that the use of ITS, LSU, *RPB2*, and *TEF1* markers provide a more accurate evaluation of their phylogenetic relationships and taxonomic placements (Hu et al. 2023; Shen et al. 2024). Most *Distoseptispora* species have been introduced based on their asexual morphs, with only two species, *D. hyalina* and *D. licualae*, described from their sexual morphs. However, the connection between sexual- and asexual morph has not yet been confirmed through cultural studies or molecular data (Yang et al. 2021; Konta et al. 2023; Liu et al. 2023).

Fungal diversity in southern China is high but remains largely unexplored. During our ongoing mycological survey of mitosporic fungi in the forest ecosystems of southwest China, several *Sporidesmium*-like taxa were isolated from dead branches of unidentified plants in Jiangxi and Fujian provinces, China. Both morphological data and multi-loci (ITS, LSU, *RPB2*, and *TEF1*) phylogenetic analyses placed six species within the genus *Distoseptispora*, including four new species, namely *D. fujianensis*, *D. ganzhouensis*, *D. nanpingensis* and *D. subtropica*, as well as two known species, viz. *D. clematidis* Phukhams., M.V. de Bult & K.D. Hyde and *D. yunjushanensis* Z.J. Zhai & D.M. Hu. Illustrations and morphological descriptions are provided, and the phylogenetic tree has been updated.

Materials and methods

Sample collection, fungal isolation, and morphological observation

Samples of dead branches were collected from forest ecosystems in Jiangxi and Fujian provinces, China, and placed in Ziploc™ bags for transport to the laboratory. The samples were treated following the methods described by Ma et al. (2011). The macroscopic structure of the colonies on the surface of the dead branches was examined and observed using a stereomicroscope (Motic SMZ-168, Xiamen, China) at magnifications ranging from low (7.5 ×) to high (50 ×). Fresh colonies were picked using sterile needles under 50 × magnification and placed on a slide with a drop of lactic acid–phenol solution (lactic acid, phenol, glycerin, sterile water; 1:1:2:1, respectively). The slide was then examined under an Olympus BX 53 light microscope equipped with an Olympus DP 27 digital camera (Olympus Optical Co., Ltd., Tokyo, Japan) to describe the microscopic morphological characteristics. A toothpick dipped in sterile water was used to collect conidia from the target colony on the surface of the dead branches. The conidia were placed on the surface of PDA (20% potato + 2% dextrose + 2% agar, w/v) and incubated overnight at 25 °C. Single germinated conidia were transferred to fresh PDA following the methods described by Goh (1999), and incubated at 25 °C. The morphological characteristics of the cultures including color, shape, and size, were observed and recorded. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. The

studied specimens and cultures were deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, China (HJAUP). The new species names were registered in Index Fungorum (Index Fungorum 2024).

DNA extraction, PCR amplification, and sequencing

Fungal hyphae were scraped with sterilized toothpicks and transferred to 2 mL safe-lock tubes, then ground with liquid nitrogen. Genomic DNA was extracted using the Solarbio Fungal Genomic DNA Extraction Kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. DNA amplification was performed by polymerase chain reaction (PCR) using the loci ITS, LSU, *RPB2*, and *TEF1*. The primer pairs used for these genes were as follows: ITS: ITS5/ITS4 (White et al. 1990), LSU: 28S1–F/28S3–R (Xia et al. 2017), *RPB2*: *RPB2*–5F2 (Sung et al. 2007) /fRPB2–7cR (Liu et al. 1999), and *TEF1*: EF1–983F/EF1–2218R (Rehner and Buckley 2005; Zhao et al. 2018). The PCR reaction was carried out in a total volume of 25 µL, containing 12.5 µL of 2 × Power Taq PCR MasterMix, 9.5 µL of double-distilled water (ddH_2O), 1 µL of DNA template, and 1 µL of each forward and reverse primer. The PCR reaction conditions for ITS, LSU, and *TEF1*, were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 54 °C for 15 s, elongation at 72 °C for 30 s, with a final extension at 72 °C for 10 min, and finally held at 4 °C. The conditions for *RBP2* were similar, except annealing was performed at 59 °C, and the elongation step was extended to 2 min. The PCR products were checked on 1% agarose gel electrophoresis stained with ethidium bromide. Purification and DNA sequencing were performed at Beijing Tsingke Biotechnology Co., Ltd., Beijing, China.

Phylogenetic analyses

The sequence datasets, comprising newly generated sequences and related sequences obtained from GenBank (Table 1), were aligned using MAFFTv.7 (Katoh and Standley 2013) on the online server (https://mafft.cbrc.jp/alignment/server/index.html, accessed on 20 April 2024), and manually optimized to improve alignment and sequence approximation. Phylogenetic analyses were carried out for each individual locus (ITS, LSU, *RPB2*, and *TEF1*), after which the four datasets were concatenated using Phylosuite software v1.2.2 (Zhang et al. 2020). The phylogenetic tree was constructed based on the concatenated ITS, LSU, *RPB2*, and *TEF1* sequence datasets using Phylosuite software v1.2.2 (Zhang et al. 2020). The concatenated and aligned datasets were analyzed separately using maximum-likelihood (ML) and Bayesian inference (BI). ML phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under an edge-linked partition model with 10,000 ultrafast bootstraps (Minh et al. 2013). The final tree was selected from suboptimal trees of each run by comparing the likelihood scores, with the SYM+I+G4 model used for ITS, TN+F+R5 for LSU and *TEF1*, and TIM3e+R3 for *RPB2*. Bayesian inference (BI) phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under a partition model (two parallel runs, 2,000,000 generations), discarding the initial 25% of sampled data as burn-in. The best-fit model was SYM+I+G4 for ITS, and GTR+F+I+G4 for LSU, *RPB2* and *TEF1*. The ModelFinder function was used to select the best

Table 1. List of *Distoseptispora* species and GenBank accession numbers used in the phylogenetic analyses.

Taxon	Strain Number	GenBank Accession Numbers			
		ITS	LSU	*RPB2*	*TEF1*
Acrodictys bambusicola	CGMCC 3.18641^{T}	KU999973	KX033564	–	–
A. elaeidicola	CGMCC 3.18642	KU999978	KX033569	–	–
Aquapteridospora aquatica	MFLUCC 17–2371^{T}	MW286493	MW287767	–	–
A. fusiformis	MFLUCC 18–1606^{T}	MK828652	MK849798	–	MN194056
A. lignicola	MFLUCC 15–0377^{T}	MZ868774	KU221018	MZ892986	MZ892980
Bullimyces aurisporus	AF316–1b^{T}	–	JF775590	–	–
B. communis	AF281–5	–	JF775587	–	–
Cancellidium applanatum	CBS 337.76^{T}	MH860985	MH872755	–	–
Cancellidium cinereum	MFLUCC 18–0424^{T}	MT370353	MT370363	MT370486	MT370488
Distoseptispora adscendens	HKUCC 10820	–	DQ408561	DQ435092	–
D. amniculi	MFLUCC 17–2129^{T}	MZ868770	MZ868761	MZ892982	–
D. appendiculata	MFLUCC 18–0259^{T}	MN163009	MN163023	–	MN174866
D. aqualignicola	KUNCC 21–10729^{T}	OK341186	ON400845	OP413474	OP413480
D. aquamyces	KUNCC 21–10732^{T}	OK341187	OK341199	OP413476	OP413482
D. aquatica	MFLUCC 18–0646	MK828648	MK849793	–	MN194052
D. aquisubtropica	GZCC 22–0075^{T}	ON527933	ON527941	ON533685	ON533677
D. arecacearum	MFLUCC 23–0212	OR354399	OR510860	OR481048	OR481045
D. atroviridis	GZCC 20–0511^{T}	MZ868772	MZ868763	MZ892984	MZ892978
D. atroviridis	GZCC 19–0531	MW133915	MZ227223	–	MZ206155
D. bambusae	MFLUCC 20–0091^{T}	MT232713	MT232718	MT232881	MT232880
D. bambusicola	GZCC 21–0667^{T}	MZ474873	MZ474872	–	OM272845
D. bangkokensis	MFLUCC 18–0262^{T}	MZ518205	MZ518206	–	OK067246
D. cangshanensis	MFLUCC 16–0970^{T}	MG979754	MG979761	–	MG988419
D. caricis	CPC 36498^{T}	MN562124	MN567632	MN556805	–
D. chinensis	GZCC 21–0665^{T}	MZ474871	MZ474867	–	MZ501609
D. clematidis	MFLUCC 17–2145^{T}	MT310661	MT214617	MT394721	–
D. clematidis	**HJAUP C1319**	**PQ211102**	**PQ211110**	**PQ303676**	**PQ303681**
D. chishuiensis	GZCC 23-0729^{T}	PP663310	PP584670	–	PP584767
D. crassispora	KUMCC 21–10726^{T}	OK310698	OK341196	OP413473	OP413479
D. curvularia	KUMCC 21–10725^{T}	OK310697	OK341195	OP413472	OP413478
D. cylindricospora	DLUCC 1906^{T}	OK491122	OK513523	–	OK524220
D. dehongensis	KUMCC 18–0090^{T}	MK085061	MK079662	–	MK087659
D. dipterocarpi	MFLUCC 22–0104^{T}	OP600053	OP600052	OP595140	–
D. effusa	GZCC 19–0532^{T}	MW133916	MZ227224	–	MZ206156
D. eleiodoxae	MFLUCC 23–0214	OR354398	OR510859	OR481047	OR481044
D. euseptata	MFLUCC 20–0154^{T}	MW081539	MW081544	MW151860	–
D. euseptata	DLUCC S2024	MW081540	MW081545	MW084996	MW084994
D. fasciculata	KUMCC 19–0081^{T}	MW286501	MW287775	–	MW396656
D. fluminicola	DLUCC 0391	MG979755	MG979762	–	MG988420
D. fluminicola	DLUCC 0999	MG979756	MG979763	–	MG988421
D. fujianensis	**HJAUP C2509^{T}**	**PQ211095**	**PQ211103**	**PQ303679**	**PQ303682**
D. fujianensis	**HJAUP C2513**	**PQ211098**	**PQ211106**	**PQ303680**	**PQ303683**
D. fusiformis	GZCC 20–0512^{T}	MZ868773	MZ868764	MZ892985	MZ892979

Taxon	Strain Number	GenBank Accession Numbers			
		ITS	LSU	*RPB2*	*TEF1*
D. ganzhouensis	**HJAUP C1090^{T}**	**PQ211100**	**PQ211108**	**–**	**PQ303687**
D. gasaensis	HJAUP C2034^{T}	OQ942896	OQ942891	–	OQ944455
D. guanshanensis	HJAUP C1063^{T}	OQ942894	OQ942898	OQ944458	OQ944452
D. guizhouensis	GZCC 21–0666^{T}	MZ474868	MZ474869	MZ501611	MZ501610
D. guttulata	MFLU 17–0852^{T}	MF077543	MF077554	–	MF135651
D. hainanensis	GZCC 22-2047^{T}	OR427328	OR438894	OR449119	OR449122
D. hyalina	MFLUCC 17–2128^{T}	MZ868769	MZ868760	MZ892981	MZ892976
D. hydei	MFLUCC 20–0481^{T}	MT734661	MT742830	MT767128	–
D. jinghongensis	HJAUP C2120^{T}	OQ942897	OQ942893	–	OQ944456
D. lancangjiangensis	DLUCC 1864^{T}	MW723055	MW879522	–	–
D. lanceolatispora	GZCC 22-2045^{T}	OR427329	OR43BB95	OR449120	OR449123
D. leonensis	HKUCC 10822	–	DQ408566	DQ435089	–
D. licualae	MFLUCC 14–1163A^{T}	ON650686	ON650675	–	ON734007
D. licualae	MFLUCC 14–1163B^{T}	ON650687	ON650676	–	ON734008
D. licualae	MFLUCC 14–1163C^{T}	ON650688	ON650677	–	–
D. lignicola	MFLUCC 18–0198^{T}	MK828651	MK849797	–	–
D. lignicola	GZCC 19–0529	MW133911	MZ227219	–	MZ206152
D. liupanshuiensis	GZCC 23-0730^{T}	PP663309	PP584669	–	PP584766
D. longispora	HFJAU 0705^{T}	MH555359	MH555357	–	–
D. longnanensis	HJAUP C1040^{T}	OQ942887	OQ942886	–	OQ944451
D. martinii	CGMCC 3.18651^{T}	KU999975	KX033566	–	–
D. meilingensis	JAUCC 4727^{T}	OK562390	OK562396	–	OK562408
D. meilingensis	JAUCC 4728^{T}	OK562391	OK562397	–	OK562409
D. menghaiensis	HJAUP C2045^{T}	OQ942890	OQ942900	–	–
D. menglunensis	HJAUP C2170^{T}	OQ942899	OQ942888	OQ944461	OQ944457
D. mengsongensis	HJAUP C2126^{T}	OP787876	OP787874	–	OP961937
D. multiseptata	MFLUCC 15–0609^{T}	KX710145	KX710140	–	MF135659
D. multiseptata	MFLU 17–0856	MF077544	MF077555	MF135644	MF135652
D. nabanheensis	HJAUP C2003^{T}	OP787873	OP787877	–	OP961935
D. nanchangensis	HJAUP C1074^{T}	OQ942889	OQ942895	OQ944460	OQ944454
D. nanpingensis	**HJAUP C2517^{T}**	**PQ211096**	**PQ211104**	**PQ303678**	**–**
D. narathiwatensis	MFLUCC 23–0216	OR354400	OR510861	OR481049	OR481046
D. neorostrata	MFLUCC 18–0376^{T}	MN163008	MN163017	–	–
D. nonrostrata	KUNCC 21–10730^{T}	OK310699	OK341198	OP413475	OP413481
D. obclavata	MFLUCC 18–0329^{T}	MN163012	MN163010	–	–
D. obpyriformis	MFLUCC 17–1694^{T}	–	MG979764	MG988415	MG988422
D. obpyriformis	DLUCC 0867	MG979757	MG979765	MG988416	MG988423
D. pachyconidia	KUMCC 21–10724^{T}	OK310696	OK341194	OP413471	OP413477
D. palmarum	MFLUCC 18–1446^{T}	MK085062	MK079663	MK087670	MK087660
D. phangngaensis	MFLUCC 16–0857^{T}	MF077545	MF077556	–	MF135653
D. phragmiticola	GUCC 220201^{T}	OP749887	OP749880	OP752699	OP749891
D. phragmiticola	GUCC 220202^{T}	OP749888	OP749881	OP752700	OP749892
D. rayongensis	MFLUCC 18–0415^{T}	MH457172	MH457137	MH463255	MH463253
D. rayongensis	MFLUCC 18–0417	MH457173	MH457138	MH463256	MH463254
D. rostrata	MFLUCC 16–0969^{T}	MG979758	MG979766	MG988417	MG988424

Taxon	Strain Number	GenBank Accession Numbers			
		ITS	LSU	*RPB2*	*TEF1*
D. rostrata	DLUCC 0885	MG979759	MG979767	–	MG988425
D. saprophytica	MFLUCC 18–1238^{T}	MW286506	MW287780	MW504069	MW396651
D. septata	GZCC 22–0078^{T}	ON527939	ON527947	ON533690	ON533683
D. sinensis	HJAUP C2044^{T}	OP787878	OP787875	–	OP961936
D. sichuanensis	KUNCC 23-15519^{T}	PP584672	PP584769	–	PP663312
D. songkhlaensis	MFLUCC 18–1234^{T}	MW286482	MW287755	–	MW396642
D. suae	CGMCC 3.24262^{T}	OQ874968	OQ732679	OQ870341	OR367670
D. subtropica	**HJAUP C2528^{T}**	**PQ211099**	**PQ211107**	**PQ303677**	**PQ303684**
D. subtropica	**HJAUP C2535**	**PQ211097**	**PQ211105**	**–**	**PQ303685**
D. suoluoensis	MFLUCC 17–0224^{T}	MF077546	MF077557	–	MF135654
D. suoluoensis	MFLUCC 17–0854	MF077547	MF077558	MZ945510	–
D. tectonae	MFLUCC 12–0291^{T}	KX751711	KX751713	KX751708	KX751710
D. tectonae	MFLU 20–0262	MT232714	MT232719	–	–
D. tectonigena	MFLUCC 12–0292^{T}	KX751712	KX751714	KX751709	–
D. thailandica	MFLUCC 16–0270^{T}	MH275060	MH260292	–	MH412767
D. thysanolaenae	KUN–HKAS 112710	MW723057	MW879524	–	MW729783
D. thysanolaenae	KUN–HKAS 102247^{T}	MK045851	MK064091	–	MK086031
D. tropica	GZCC 22–0076^{T}	ON527935	ON527943	ON533687	ON533679
D. verrucosa	GZCC 20–0434^{T}	MZ868771	MZ868762	MZ892983	MZ892977
D. wuzhishanensis	GZCC 22–0077^{T}	ON527938	ON527946	–	ON533682
D. xinpingensis	KUNCC 22–12669	OQ874969	OQ732680	–	–
D. xinpingensis	KUNCC 22–12667^{T}	OQ874970	OQ732681	OQ870340	OR367671
D. xishuangbannaensis	KUMCC 17–0290^{T}	MH275061	MH260293	MH412754	MH412768
D. yichunensis	HJAUP C1065^{T}	OQ942885	OQ942892	OQ944459	OQ944453
D. yongxiuensis	JAUCC 4725^{T}	OK562388	OK562394	–	OK562406
D. yongxiuensis	JAUCC 4726^{T}	OK562389	OK562395	–	OK562407
D. yunjushanensis	JAUCC 4723^{T}	OK562392	OK562398	–	OK562410
D. yunjushanensis	JAUCC 4724^{T}	OK562393	OK562399	–	OK562411
D. yunjushanensis	**HJAUP C1307**	**PQ211101**	**PQ211109**	**PQ303675**	**PQ303686**
D. yunnansis	MFLUCC 20–0153^{T}	MW081541	MW081546	MW151861	MW084995
Fluminicola saprophytica	MFLUCC 15–0976^{T}	MF374358	MF374367	MF370954	MF370956
Myrmecridium banksiae	CPC 19852 = CBS 132536^{T}	JX069871	JX069855	–	–
M. schulzeri	CBS 100.54	EU041769	EU041826	–	–
Papulosa amerospora	AFTOL–ID 748	–	DQ470950	DQ470901	DQ471069
Pleurophragmium bambusinum	MFLUCC 12–0850	KU940161	KU863149	–	KU940213
Pseudostanjehughesia aquitropica	MFLUCC 16–0569^{T}	MF077548	MF077559	–	MF135655
P. lignicola	MFLUCC 15–0352^{T}	MK828643	MK849787	MN124534	MN194047
Wongia griffinii	DAR 80512^{T}	KU850473	KU850471	–	–

Notes: The ex-type cultures are indicated using "^{T}" after strain numbers; "—" stands for no sequence data in GenBank. **AFTOL**: Assembling the Fungal Tree of Life; **CBS**: Central Bureau voor Schimmel cultures, Utrecht, The Netherlands; **CGMCC**: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; **CPC**: Collection of P.W. Crous; **DAR**: Plant Pathology Herbarium, Orange Agriculture Institute, NSW, Australia; **DLUCC**: Dali University Culture Collection, Yunnan; **GZCC**: Guizhou Culture Collection China; **HFJAU**: Herbarium of Fungi, Jiangxi Agricultural University; **HKUCC**: The University of Hong Kong Culture Collection, Hong Kong, China; **JAUCC**: Jiangxi Agricultural University Culture Collection; **KUMCC**: Kunming Institute of Botany Culture Collection; **HKAS**: Kunming Institute of Botany Academia Sinica, Yunnan, China; **MFLU**: the herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

fitting partition model (Edge-linked) (Kalyaanamoorthy et al. 2017), using the BIC criterion for constructing IQ-TREE and the AICc criterion for MrBayes. These trees were visualized using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree, accessed on 8 September 2024), with further editing and composition performed in Adobe Illustrator CS v. 5. The sequences generated in this study were deposited in GenBank.

Results

Molecular phylogeny

Based on the combined sequences of ITS, LSU, *RPB2* and *TEF1*, eight strains from the present study were analyzed for their phylogenetic relationships within the family Distoseptisporaceae and related families (Acrodictyaceae, Aquapteridosporaceae, Bullimycetaceae, Cancellidiaceae, Papulosaceae, and Pseudostanjehughesiaceae), using 126 strains representing 104 species (Table 1). The combined sequence alignment consisted of 2841 columns (ITS:1–579, LSU:580–1372, *RPB2*:1373–2011, *TEF1*:2012–2841), including 1455 distinct patterns, 1124 parsimony-informative sites, 271 singleton sites, and 1446 constant sites. *Myrmecridium banksiae* (CBS 132536) and *M. schulzeri* (CBS 100.54) were used as the outgroup. Maximum-likelihood (ML) and Bayesian inference (BI) analyses of the combined sequences resulted in a phylogenetic reconstruction with essentially similar topological structures. The best-scoring ML tree (lnL = -38006.722) with ultrafast bootstrap values from ML analyses and posterior probabilities from MrBayes analysis are shown in the first and second values above the nodes in Fig. 1. Based on multilocus phylogeny and morphology, the eight strains in this study were assigned to four new species, namely *D. fujianensis*, *D. ganzhouensis*, *D. nanpingensis*, and *D. subtropica*, and two known species, viz. *D. clematidis*, and *D. yunjushanensis*.

Taxonomy

Distoseptispora clematidis **Phukhams., M.V. de Bult & K.D. Hyde, Fungal Diversity 102: 168 (2020)**

Index Fungorum: IF557301
Facesoffungi Number: FoF07261
Fig. 2

Description. ***Saprobic*** on dead branches in a terrestrial habitat. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, dark brown to black, hairy. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, pale brown to brown hyphae. ***Conidiophores*** macronematous, mononematous, unbranched, solitary or in groups, erect, straight or slightly flexuous, cylindrical, smooth, brown to dark brown, 2–5-septate, robust at the base, 25–48.5 × 5–8 µm ($\bar{x}$ = 34.9 × 6.1 µm, n = 12). ***Conidiogenous cells*** monoblastic, integrated, terminal, cylindrical, pale brown to brown, smooth, flat at the conidiogenous loco. ***Conidia*** acrogenous, solitary, dry, obclavate, straight or curved, reddish-brown and slightly paler towards the apex, 26–40-distoseptate, smooth,

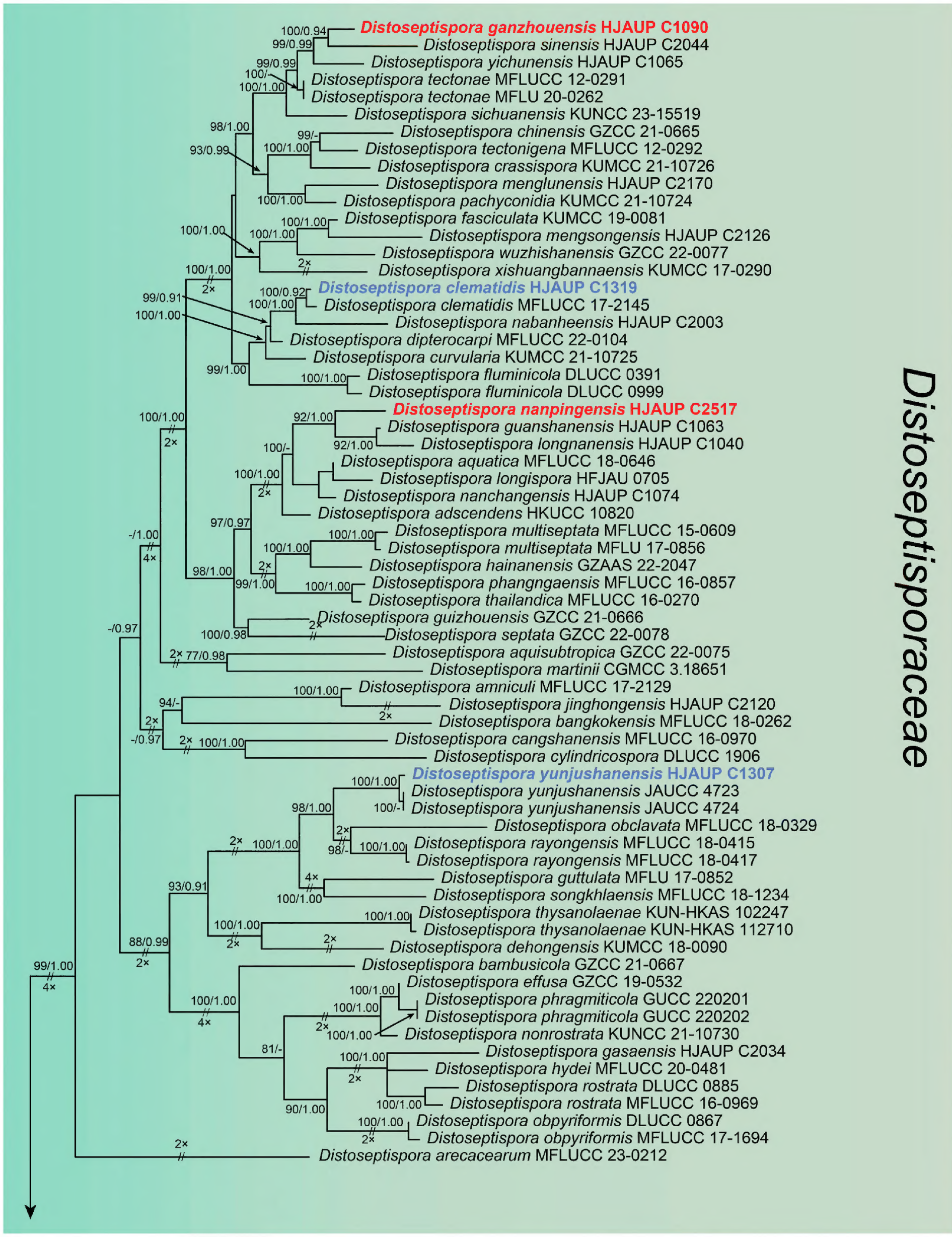
Distoseptispora ganzhouensis HJAUP C1090
Distoseptispora sinensis HJAUP C2044
Distoseptispora yichunensis HJAUP C1065
Distoseptispora tectonae MFLUCC 12-0291
Distoseptispora tectonae MFLU 20-0262
Distoseptispora sichuanensis KUNCC 23-15519
Distoseptispora chinensis GZCC 21-0665
Distoseptispora tectonigena MFLUCC 12-0292
Distoseptispora crassispora KUMCC 21-10726
Distoseptispora menglunensis HJAUP C2170
Distoseptispora pachyconidia KUMCC 21-10724
Distoseptispora fasciculata KUMCC 19-0081
Distoseptispora mengsongensis HJAUP C2126
Distoseptispora wuzhishanensis GZCC 22-0077
Distoseptispora xishuangbannaensis KUMCC 17-0290
Distoseptispora clematidis HJAUP C1319
Distoseptispora clematidis MFLUCC 17-2145
Distoseptispora nabanheensis HJAUP C2003
Distoseptispora dipterocarpi MFLUCC 22-0104
Distoseptispora curvularia KUMCC 21-10725
Distoseptispora fluminicola DLUCC 0391
Distoseptispora fluminicola DLUCC 0999
Distoseptispora nanpingensis HJAUP C2517
Distoseptispora guanshanensis HJAUP C1063
Distoseptispora longnanensis HJAUP C1040
Distoseptispora aquatica MFLUCC 18-0646
Distoseptispora longispora HFJAU 0705
Distoseptispora nanchangensis HJAUP C1074
Distoseptispora adscendens HKUCC 10820
Distoseptispora multiseptata MFLUCC 15-0609
Distoseptispora multiseptata MFLU 17-0856
Distoseptispora hainanensis GZAAS 22-2047
Distoseptispora phangngaensis MFLUCC 16-0857
Distoseptispora thailandica MFLUCC 16-0270
Distoseptispora guizhouensis GZCC 21-0666
Distoseptispora septata GZCC 22-0078
Distoseptispora aquisubtropica GZCC 22-0075
Distoseptispora martinii CGMCC 3.18651
Distoseptispora amniculi MFLUCC 17-2129
Distoseptispora jinghongensis HJAUP C2120
Distoseptispora bangkokensis MFLUCC 18-0262
Distoseptispora cangshanensis MFLUCC 16-0970
Distoseptispora cylindricospora DLUCC 1906
Distoseptispora yunjushanensis HJAUP C1307
Distoseptispora yunjushanensis JAUCC 4723
Distoseptispora yunjushanensis JAUCC 4724
Distoseptispora obclavata MFLUCC 18-0329
Distoseptispora rayongensis MFLUCC 18-0415
Distoseptispora rayongensis MFLUCC 18-0417
Distoseptispora guttulata MFLU 17-0852
Distoseptispora songkhlaensis MFLUCC 18-1234
Distoseptispora thysanolaenae KUN-HKAS 102247
Distoseptispora thysanolaenae KUN-HKAS 112710
Distoseptispora dehongensis KUMCC 18-0090
Distoseptispora bambusicola GZCC 21-0667
Distoseptispora effusa GZCC 19-0532
Distoseptispora phragmiticola GUCC 220201
Distoseptispora phragmiticola GUCC 220202
Distoseptispora nonrostrata KUNCC 21-10730
Distoseptispora gasaensis HJAUP C2034
Distoseptispora hydei MFLUCC 20-0481
Distoseptispora rostrata DLUCC 0885
Distoseptispora rostrata MFLUCC 16-0969
Distoseptispora obpyriformis DLUCC 0867
Distoseptispora obpyriformis MFLUCC 17-1694
Distoseptispora arecacearum MFLUCC 23-0212
Distoseptisporaceae

Figure 1. Maximum-likelihood majority rule consensus tree for Distoseptisporaceae and related families using ITS, LSU, *RPB2*, and *TEF1* sequence data. Bootstrap support values for maximum-likelihood greater than 75% and Bayesian posterior probabilities greater than 0.90 are shown near the nodes. The tree is rooted with *Myrmecridium schulzeri* (CBS 100.54) and *M. banksiae* (CBS 132536). New strains identified in this study are shown in blue; new species are shown in red. Some branches were shortened according to the indicated multipliers, and these are indicated by the symbol (//).

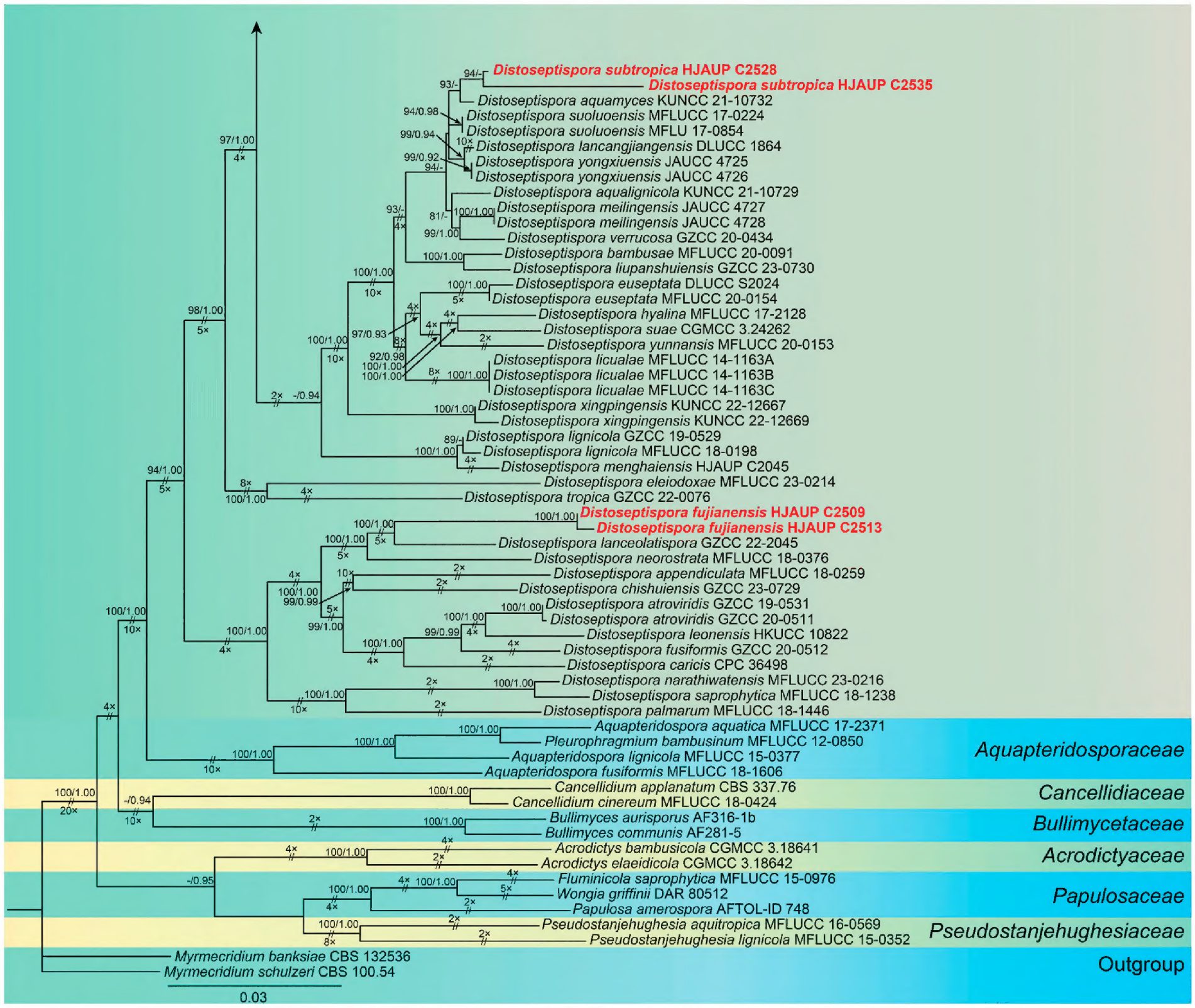

Figure 1. Continued.

150–270 × 11–15 µm ($\bar{x}$ = 195.1 × 12 µm, n= 30), tapering to 2.5–7.5 µm near the apex, 2.5–5.5 µm wide at the truncate base.

Culture characteristics. ***Colonies*** on PDA reaching 76–80 mm diam. after 4 weeks in an incubator under dark conditions at 25 °C, circular, surface velvety, with brown and denser mycelium at the center, becoming black at the margin with an obvious boundary, reverse black and sparser toward the boundary.

Material examined. China • Jiangxi Province, Ganzhou City, Jiulianshan National Nature Reserve, 24°31'N, 114°27'E, on decaying wood of an unidentified broadleaf tree, 29 June 2022, Y.F. Hu (HJAUP M1319, living culture HJAUP C1319).

Notes. *Distoseptispora clematidis* was introduced by Phukhamsakda et al. (2020), isolated from the dried stem of *Clematis sikkimensis* in Thailand. Phylogenetic analysis shows that our new isolate (HJAUP C1319) clusters with *D. clematidis* Phukhams., M.V. de Bult & K.D. Hyde (MFLUCC 17–2145), with 100% ML/0.92 BI support. Morphologically, our isolate aligns well with the holotype description of *D. clematidis*. Comparisons of their nucleotide sequences showed 4 (0.7%, including one gap), 3 (0.6%, no gaps), and 2 (0.3%, no gaps) nucleotide differences in the ITS, LSU, and *RPB2* regions, respectively, indicating that they are the same species.

Figure 2. *Distoseptispora clematidis* (HJAUP M1319) **a** surface of colony after 4 weeks on PDA **b** reverse of colony after 4 weeks on PDA **c** conidiophores, conidiogenous cells and conidia **d, e** conidia **f** conidiophores.

Distoseptispora fujianensis **M.G. Liao & Jian Ma, sp. nov.**

Index Fungorum: IF902665

Fig. 3

Type. CHINA • Fujian Province, Nanping City, Wuyishan National Nature Reserve, 27°43'N, 117°41'E, on decaying wood of an unidentified broadleaf tree, 16 October 2023, Y.F. Hu (holotype HJAUP M2509; ex-type living culture HJAUP C2509).

Etymology. In reference to Fujian Province, where the fungus was collected.

Description. ***Saprobic*** on dead branches in a terrestrial habitat. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, dark brown to black, hairy. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, pale brown to brown hyphae. ***Conidiophores*** macronematous, mononematous, unbranched, solitary or in groups of 2 or 3, straight or slightly flexuous, cylindrical, 6–9-septate, brown to dark brown, 86–127 × 4.5–6 μm ($\bar{x}$ = 103.4 × 5.2 μm, n = 11). ***Conidiogenous cells*** monoblastic, integrated, terminal, cylindrical, brown, smooth, determinate, flat at the conidiogenous loco. ***Conidia*** solitary, acroge-

Figure 3. *Distoseptispora fujianensis* (HJAUP M2509, holotype) **a** surface of colony after 4 weeks on PDA **b** reverse of colony after 4 weeks on PDA **c** conidia **d, e** conidiophores, conidiogenous cells and conidia **f** conidiophores.

nous, dry, obclavate or ellipsoidal, smooth, 6–8-distoseptate, brown, apical cell paler, 28–47 × 8–14 µm ($\bar{x}$ = 37.7 × 10.3 µm, n = 23), tapering to 4.5–9 µm near the apex, 4.5–7 µm wide at the truncate base.

Culture characteristics. ***Colonies*** on PDA reaching 60–63 mm diam. after 4 weeks in an incubator under dark conditions at 25 °C, circular, surface velvety, with pale brown and denser mycelium and the black edge, reverse black.

Additional specimen examined. China • Fujian Province, Nanping City, Wuyishan National Nature Reserve, 27°43'N, 117°41'E, on decaying wood of an unidentified broadleaf tree, 16 October 2023, Y.F. Hu (paratype HJAUP M2513, living culture HJAUP C2513).

Notes. Phylogenetic analyses shows that two strains of *D. fujianensis* (HJAUP C2509 and HJAUP C2513) form a highly support clade, sister to *D. lanceolatispora* X.M. Chen & Y.Z. Lu (GZCC 22-2045), with 100% ML/1.00 BI support. A BLASTn search of GenBank reveals that the sequences of *D. fujianensis* (HJAUP C2509) and *D. lanceolatispora* (GZCC 22-2045) share 96% similarity (504/527, 9 gaps) in ITS, 99% similarity (567/572, 1 gap) in LSU, 92% similarity (990/1075, no gaps) in *RPB2*, and 98% similarity (884/906, no gaps) in *TEF1*. Moreover, *D. fujianensis* differs from *D. lanceolatispora* (Chen et al. 2024) by having shorter conidiophores (86–127 µm vs. 120–190 µm) and smaller conidia (28–47 × 8–14 µm vs. 31–90 × 9.5–15 µm). In addition, *D. fujianensis* further differs from *D. lanceolatispora* by its terrestrial habitat, as opposed to the freshwater habitat of *D. lanceolatispora*.

Distoseptispora ganzhouensis **M.G. Liao & Jian Ma, sp. nov.**

Index Fungorum: IF902666

Fig. 4

Type. CHINA • Jiangxi Province, Ganzhou City, Jiulianshan National Nature Reserve, 24°31'N, 114°27'E, on decaying wood of an unidentified broadleaf tree, 29 June 2022, Y.F. Hu (holotype HJAUP M1090; ex-type living culture HJAUP C1090).

Etymology. In reference to the locality, Ganzhou city, where the fungus was collected.

Description. ***Saprobic*** on dead branches in a terrestrial habitat. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. ***Colonies*** on natural substratum effuse, hairy, and olivegreen to pale brown. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, pale brown to brown hyphae. ***Conidiophores*** macronematous, mononematous, solitary, unbranched, straight or flexuous, smooth, 5–10 septate, cylindrical, brown to dark brown, paler towards the apex, determinate or sometimes with a cylindrical, enteroblastic percurrent extension, 54–93 × 4.5–7 µm, ($\bar{x}$ = 73.1 × 6.2 µm, n = 15). ***Conidiogenous cells*** monoblastic, integrated, terminal, cylindrical, pale brown to brown, smooth, flat at the conidiogenous loco. ***Conidia*** solitary, acrogenous,

Figure 4. *Distoseptispora ganzhouensis* (HJAUP M1090, holotype) **a** surface of colony after 4 weeks on PDA **b** reverse of colony after 4 weeks on PDA **c** conidiophores **d, e** conidiophores, conidiogenous cells and conidia **f, g** conidia.

obclavate, straight or curved, pale brown to brown, 10–23-distoseptate, smooth, 59–139 × 11.5–16.5 µm ($\bar{x}$ = 96.4 × 13.6 µm, n = 30), tapering to 4–8.5 µm near the apex, 3–6 µm wide at the truncate base.

Culture characteristics. ***Colonies*** on PDA reaching 54 mm diam. after 4 weeks in an incubator under dark conditions at 25 °C, circular, surface velvety, with dense and brown mycelium, dark brown to pale at the margin, reverse dark brown to black.

Notes. Phylogenetic analyses shows that *D. ganzhouensis* (HJAUP C1090) clusters with *D. sinensis* Jing W. Liu, X.G. Zhang & Jian Ma (HJAUP C2044), with 100% ML/0.94 BI support. A BLASTn search of GenBank reveals that the sequences of *D. ganzhouensis* (HJAUP C1090) and *D. sinensis* (HJAUP C2044) share 98% similarity (555/565, one gaps) in ITS, 99% similarity (557/559, two gaps) in LSU, and 99% similarity (925/930, no gaps) in *TEF1*. Moreover, *D. ganzhouensis* is significantly different from *D. sinensis* (Liu et al. 2023) by having longer conidiophores (54–93 µm vs. 23.5–56.5 µm) and wider conidia (11.5–16.5 µm vs. 10–12 µm).

Distoseptispora nanpingensis **M.G. Liao & Jian Ma, sp. nov.**

Index Fungorum: IF902669

Fig. 5

Type. China • Fujian Province, Nanping City, Wuyishan National Nature Reserve, 27°43'N, 117°41'E, on decaying wood of an unidentified broadleaf tree, 16 October 2023, Y.F. Hu (holotype HJAUP M2517; ex-type living culture HJAUP C2517).

Etymology. In reference to the locality, Nanping city, where the fungus was collected.

Description. ***Saprobic*** on dead branches in a terrestrial habitat. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, dark brown to black, hairy. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, pale brown to brown hyphae. ***Conidiophores*** macronematous, mononematous, unbranched, solitary, straight or slightly flexuous, cylindrical, smooth, 1–3-septate, brown to dark brown, 8.5–28 × 5–7 µm ($\bar{x}$ = 18.1 × 6.1 µm, n = 10). ***Conidiogenous cells*** monoblastic, integrated, terminal, determinate, cylindrical, flat at the conidiogenous loco. ***Conidia*** solitary, acrogenous, dry, obclavate, straight or curved, sometimes with a swollen cell, reddish-brown and slightly paler towards the apex, 28–41-distoseptate, sometimes constricted at the septa, smooth, sometimes with percurrent regeneration forming a secondary conidium from the conidial apex, 169–282 × 12–17.5 µm ($\bar{x}$ = 227.1 × 14.8 µm, n = 20), tapering to 5–9 µm near the apex, 3.5–5.5 µm wide at the truncate base.

Culture characteristics. ***Colonies*** on PDA reaching 67–71 mm diam. after 4 weeks in an incubator under dark conditions at 25 °C, irregularly circular, surface velvety with white to pale brown and denser mycelium, becoming black at the margin, reverse black.

Notes. Phylogenetic analyses shows that *D. nanpingensis* (HJAUP C2517) forms a distinct clade, sister to the clade containing *D. guanshanensis* Y. F. Hu & Jian Ma (HJAUP C1063) and *D. longnanensis* Y.F. Hu & Jian Ma (HJAUP C1040), with 92% ML/1.00 BI support. A BLASTn search of GenBank reveals that the

Figure 5. *Distoseptispora nanpingensis* (HJAUP M2517, holotype) **a** surface of colony after 4 weeks on PDA **b** reverse of colony after 4 weeks on PDA **c, d** conidiophores, conidiogenous cells and conidia **e, f** Conidia.

sequences of *D. nanpingensis* (HJAUP C2517) and *D. guanshanensis* (HJAUP C1063) share 99% similarity (602/608, no gaps) in ITS, 99% similarity (546/547, no gaps) in LSU, 98% similarity (906/923, four gaps) in *RPB2*, and 98% similarity (906/927, no gaps) in *TEF1*. The sequences of *D. longnanensis* (HJAUP C1040) share 98% similarity (608/621, five gaps) in ITS, 99% similarity (560/565, two gaps) in LSU, and 98% similarity (903/923, no gaps) in *TEF1*. Moreover, *D. nanpingensis* is significantly different from *D. guanshanensis* (Hu et al. 2023) by having shorter conidiophores (8.5–28 μm vs. 15.4–44.7 μm), and longer conidia (169–282 μm vs. 96.5–255.3 μm) sometimes with percurrent regeneration forming a secondary conidium from the conidial apex. *Distoseptispora nanpingensis* also differs from *D. longnanensis* (Hu et al. 2023) by having shorter conidiophores (8.5–28 μm vs. 77–171 μm) and larger conidia (169–282 × 12–17.5 μm vs. 54–87 × 8.2–14 μm) with more distosepta (28–41 vs. 4–8).

Distoseptispora subtropica **M.G. Liao & Jian Ma, sp. nov.**

Index Fungorum: IF902670

Fig. 6

Type. China • Fujian Province, Nanping City, Wuyishan National Nature Reserve, 27°43'N, 117°41'E, on decaying wood of an unidentified broadleaf tree, 16 October 2023, YF. Hu (holotype HJAUP M2528; ex-type living culture HJAUP C2528).

Figure 6. *Distoseptispora subtropica* (HJAUP M2528, holotype) **a** surface of colony after 4 weeks on PDA **b** reverse of colony after 4 weeks on PDA **c–e** conidiophores, conidiogenous cells and conidia **f** conidia **g** conidiophores.

Etymology. In reference to the subtropical climate in which the species was collected.

Description. ***Saprobic*** on dead branches in a terrestrial habitat. **Sexual morph**: Undetermined. **Asexual morph**: Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, dark brown to black, hairy. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, pale brown to brown hyphae. ***Conidiophores*** macronematous, mononematous, solitary or in groups, unbranched, erect, straight or slightly flexuous, cylindrical, smooth, 5–10-septate, brown to dark brown and paler towards the apex, 125–262 × 6–10.5 μm ($\bar{x}$ = 181.7 × 6.7 μm, n = 12). ***Conidiogenous cells*** monoblastic or ployblastic, integrated, terminal, cylindrical, pale brown, smooth, determinate or sometimes with cylindrical, enteroblastic percurrent extensions. ***Conidia*** acropleurogenous, solitary, dry, straight or slightly curved, obclavate, rostrate, verrucose, 5–10-euseptate, pale brown to brown, 64–147 × 12–15 μm ($\bar{x}$ = 90.1 × 13.3 μm, n = 23), tapering to 2.5–4.5 μm near the apex, 4.5–7 μm wide at the truncate base.

Culture characteristics. ***Colonies*** on PDA reaching 57–58 mm diam. after 4 weeks in an incubator under dark conditions at 25 °C, circular, surface velvety, with yellow to pale brown and denser mycelium at the center, becoming dark brown at the ring with a pale edge, reverse brown at the central parts with a dark brown inner ring and an outer pale brown halo.

Additional specimen examined. China • Fujian Province, Nanping City, Wuyishan National Nature Reserve, 27°43'N, 117°41'E, on decaying wood of an

unidentified broadleaf tree, 16 October 2023, Y.F. Hu (paratype HJAUP M2535; living culture HJAUP C2535).

Notes. Strains HJAUP C2528 and HJAUP C2535 grouped together with 94% ML/0.81 BI support. Comparisons of their nucleotide sequences revealed differences of 12 nucleotides (1.9%, including five gaps) in the ITS region, 7 nucleotides (1.2%, including three gaps) in the LSU region, and 13 nucleotides (1.3%, including two gaps) in the *TEF1* region. Based on the criteria established by Jeewon and Hyde (2016), nucleotide differences greater than 1.5% in the ITS region may suggest a new species. However, aside from slight differences in conidial length (64–147 µm vs. 53–86 µm), there were no significant morphological distinctions. Therefore, we propose to identify these two strains as the same new species, *Distoseptispora subtropica*. Phylogenetic analyses show that *D. subtropica* (HJAUP C2528 and HJAUP C2535) forms a sister clade to *D. aquamyces* R. Zhu & H. Zhang (KUNCC 21–10732), with 93% ML/0.81 BI support. A BLASTn search of GenBank reveals that the sequences of *D. subtropica* (HJAUP C2528) and *D. aquamyces* (KUNCC 21–10732) share 99% similarity (568/575, two gaps) in ITS, 99% similarity (571/574, three gaps) in LSU, 99% similarity (1035/1040, three gaps) in *RPB2*, and 99% similarity (912/919, no gaps) in *TEF1*. Moreover, *D. subtropica* is significantly different from *D. aquamyces* (Zhang et al. 2022) by having larger conidiophores [125–262 × 6–10.5 µm vs. (78–)91–198 × 4–7 µm], and acropleurogenous, obclavate, larger conidia (64–147 × 12–15 µm vs. 30–95 × 7–12 µm).

***Distoseptispora yunjushanensis* Z.J. Zhai & D.M. Hu, MycoKeys 88: 47 (2022)**

MycoBank No: 842065

Fig. 7

Description. ***Saprobic*** on dead branches in a terrestrial habitat. **Sexual morph**: Undetermined. **Asexual morph**: Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, dark brown to black, hairy. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, pale brown to brown hyphae. ***Conidiophores*** macronematous, mononematous, solitary or in groups, unbranched, erect, straight or slightly flexuous, cylindrical, smooth, 3–6-septate, pale brown to brown, 125–243 × 7.5–10.5 µm ($\bar{x}$ = 185 × 9.9 µm, n = 14). ***Conidiogenous cells*** monoblastic, integrated, terminal, determinate or sometimes with one cylindrical percurrent extension, cylindrical, pale brown, smooth. ***Conidia*** acrogenous, solitary, dry, obclavate or ellipsoidal, 5–10-distoseptate, smooth, brown, paler toward the apex, 67.5–96 × 16–22.5 µm ($\bar{x}$ = 83.2 × 19.4 µm, n = 30), tapering to 2.5–12.5 µm near the apex, 5–7.5 µm wide at the truncate base.

Culture characteristics. ***Colonies*** on PDA reached 65–75 mm diam. after 4 weeks in an incubator under dark conditions at 25 °C, circular, surface velvety, dark brown at the center, with dense, brown mycelium, black margin entire with flocculous mycelium, reverse black.

Material examined. CHINA • Jiangxi Province, Ganzhou City, Jiulianshan National Nature Reserve, 24°31'N, 114°27'E, on decaying wood of an unidentified broadleaf tree, 29 June 2022, Y.F. Hu (HJAUP M1307, living culture HJAUP C1307).

Notes. *Distoseptispora yunjushanensis*, introduced by Zhai et al. (2022), was originally found on decaying bamboo culms submerged in a freshwater stream

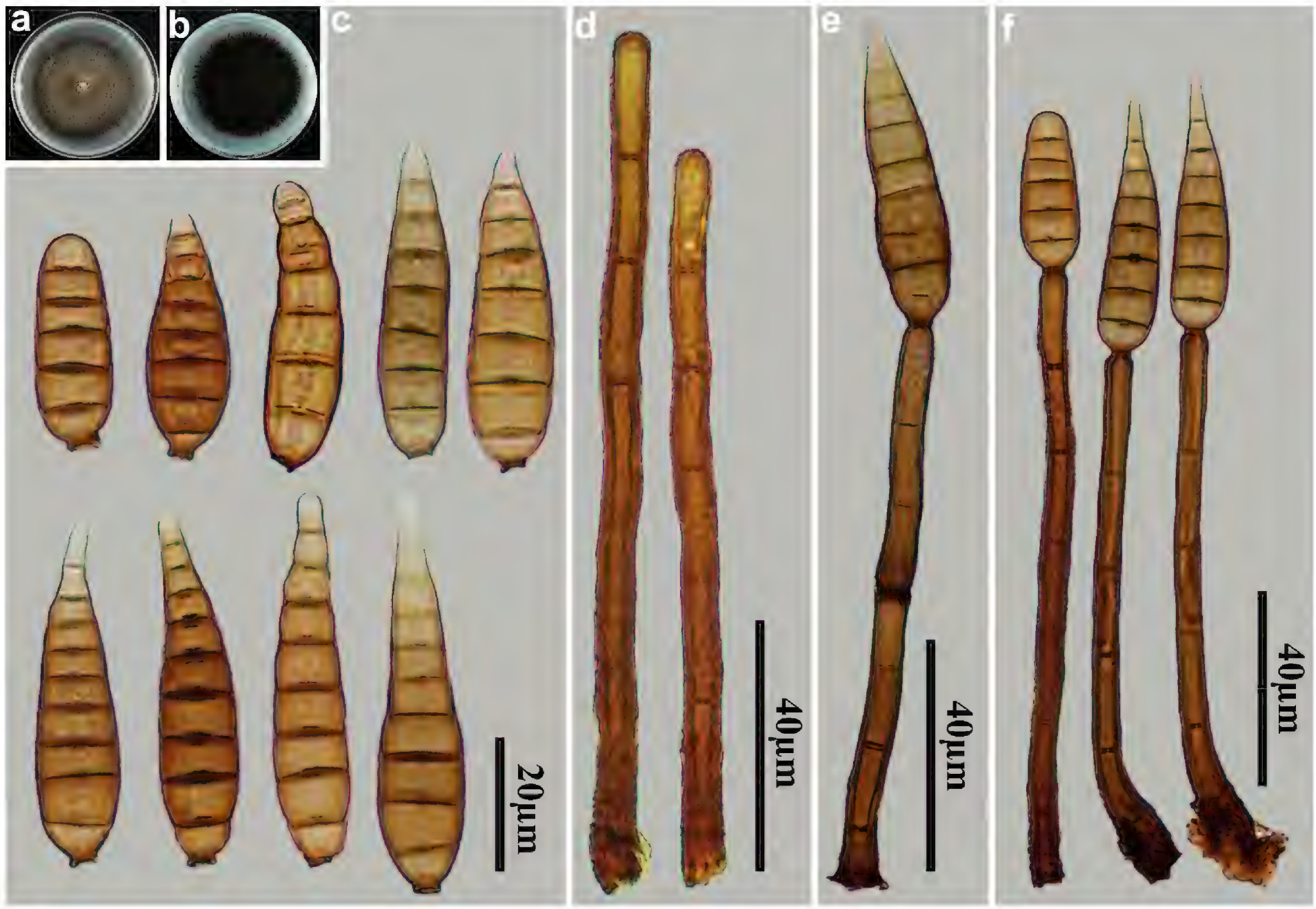

Figure 7. *Distoseptispora yunjushanensis* (HJAUP M1307) **a** surface of colony after 4 weeks on PDA **b** reverse of colony after 4 weeks on PDA **c** conidia **d** conidiophores **e, f** conidiophores, conidiogenous cells and conidia.

in China. Phylogenetic analysis shows that our new isolate (HJAUP C1307) clusters with *D. yunjushanensis* Z. J. Zhai & D. M. Hu (JAUCC 4723 and JAUCC 4724) with 100% ML/1.00 BI support. Comparisons of nucleotide sequences for our new isolate (HJAUP C1307) and *D. yunjushanensis* (JAUCC 4723) showed 7 (1.3%, including three gaps) and 3 (0.5%, including one gap) nucleotide differences in the ITS and LSU regions, respectively. Morphologically, our isolate fits well with the holotype description of *D. yunjushanensis*, except for its longer conidiophores (125–242.5 μm vs. 100–175 μm) and larger conidia [67.5–96 × 16–22.5 μm vs. 39–67.5(–77) × (7–)9.5–13.5(–16.5) μm] with fewer distosepta (4–10 vs. 7–13). In addition, our isolate was collected from a terrestrial habitat, as opposed to the freshwater habitat of the holotype of *D. yunjushanensis*. However, due to the high morphological similarity and only minor molecular differences, our new isolate does not meet the criteria for a new species, and is thus identified as *D. yunjushanensis*.

Discussion

Distoseptispora is one of the sporidesmium-like genera introduced by Su et al. (2016) based on molecular phylogenetic analyses combined with morphology. It exhibits a conidial ontogeny similar to that of *Sporidesmium* Link and *Ellisembia* Subram., which produce solitary, holoblastic conidia on unbranched conidiophores that may undergo percurrent proliferation. The excessive overlap in morphological features makes it difficult to distinguish *Distoseptispora* from

Sporidesmium and *Ellisembia* based solely on morphology, however, these genera are not closely related in phylogenetic trees (Su et al. 2016; Yang et al. 2018; Luo et al. 2019). In addition, *Distoseptispora* forms a distinct and well-supported clade sister to *Aquapteridospora* Jiao Yang, K.D. Hyde & Maharachch., which produces 3-euseptate conidia on terminal and intercalary conidiogenous cells with circular scars (Yang et al. 2015; Hyde et al. 2021). Both *Distoseptispora* and *Aquapteridospora* are placed in Distoseptisporales, but are treated as separate families, Distoseptisporaceae K.D. Hyde & McKenzie and Aquapteridosporaceae K.D. Hyde & Hongsanan (Wijayawardene et al. 2020; Hyde et al. 2021).

In recent years, the number of *Distoseptispora* species has steadily increased, now reaching 87 valid taxa, including the four species described in this study. All species have been identified based on morphological and phylogenetic analyses. Liu et al. (2023) listed 63 recognized *Distoseptispora* species with major morphological features, habitat, host, and locality. Subsequently, 24 additional species have been added to the genus, and they are presented in Table 2, following the same format as Liu et al. (2023). *Distoseptispora* species are typically saprobic fungi on dead plant material in freshwater and terrestrial habitats, with the exception of *D. caricis* and *D. palmarum*, which occur on the leaves of *Carex* sp. and the rachis of *Cocos nucifera*, respectively (Crous et al. 2019; Hyde et al. 2019; Hu et al. 2023; Liu et al. 2023; Karimi et al. 2024; Shen et al. 2024). Some *Distoseptispora* species show morphological variation across different specimens, but they can be reliably identified through molecular DNA data. For instance, the conidiophores of *D. tectonae* Doilom & K.D. Hyde are up to 40 × 4–6 µm in the holotype (MFLU 15-3417) (Hyde et al. 2016), but 34–95 × 5–8 µm in a later collection (MFLU 20–0262) (Sun et al. 2020). The conidia of *D. pachyconidia* are 42–136 × 14–22 µm, 8–21-distoseptate, pale-brown with a green tinge in the holotype (HKAS 122179) (Zhang et al. 2022), but 50–242 × 11–20 µm, up to 38-distoseptate, olivaceous to dark brown in another collection (HKAS 123754) (Ma et al. 2022), and (82–)137–246(–296) × (9–)13–16 µm, 14–45-distoseptate, pale brown to brown in yet another collection (HKAS 125824) (Shen et al. 2024). These variations may result from differences in geographic regions, ecological environments, climatic conditions, substrates and habitats, or observation periods. Although some *Distoseptispora* species, such as *D. bambusae*, *D. pachyconidia*, and *D. tectonae*, show morphological variation from the holotype, the BLASTn analysis of multilocus gene sequences shows only minor differences. The use of ITS, LSU, *RPB2* and *TEF1* loci provides a more reliable evaluation of their phylogenetic relationships and taxonomic placements.

At present, molecular data have greatly advanced the development of fungal taxonomy and phylogenetic relationships, leading to taxonomic revisions and the identification of new species (Naranjo-Ortiz and Gabaldón 2020). While *Distoseptispora* species share morphological similarities within this genus or with other sporidesmium-like taxa, molecular phylogenetic analyses offer strong support for determining their taxonomic status. However, there are no universally accepted criteria for selecting loci for phylogenetic analysis in *Distoseptispora* (Liu et al. 2023), although analyses using ITS, LSU, *RPB2* and *TEF1* loci have yielded more accurate evaluations of phylogenetic relationships and taxonomic placements. Nevertheless, some inconsistencies remain in the morphological characteristics of the same phylogenetic species. For example, the conidiogenous cells of *D. bambusae* are blastic in the holotype (MFLU 20–0261) collected from

Table 2. Synopsis of morphological characteristics, habitat, host, and locality compared across *Distoseptispora* species described since Liu et al. (2023).

Species	Conidiophores (µm)	Conida		Habitat	Host/Locality	References
		Size (µm)	Morphology			
Distoseptispora arecacearum	70–140 × 5.1–6.3	25–60 × 7–17	Cylindrical, obclavate to obpyriform or irregular, brown, sometimes with percurrent regeneration forming a secondary conidium from the conidial apex, 4–10-distoseptate	Freshwater	Submerged rachis of *Licuala paludosa*, Thailand	Karimi et al. (2024)
D. chishuiensis	80–190 × 4–7	70–130 × 14.5–21	Obpyriform or obclavate, thick-walled, olivaceous or dark brown below, hyaline towards apex, slender and rounded at apex, 13–17-distoseptate	Terrestrial	Dead culms of bamboo, China	Dissanayake et al. (2024)
D. dipterocarpi	14–72 × 5–7	31.5–350 × 6.5–12	Cylindrical or obclavate, elongated, olivaceous when young, brown tinge when mature, mostly lighter towards the apex, 10–72-distoseptate	Terrestrial	Woody litter of *Dipterocarpus*, Thailand	Afshari et al. (2023)
D. eleiodoxae	71–161 × 5–6.5	31.5–48 × 13.5–15.8	Obpyriform, rostrate, verrucose, brown with dark brown to black cells in the middle, paler towards the apex, 6–7-euseptate	Freshwater	Submerged rachis of *Eleiodoxa conferta*, Thailand	Karimi et al. (2024)
D. fujianensis	86–127 × 4.5–6	28–47 × 8–14	obclavate or ellipsoidal, brown, apical cell paler, 6–8-distoseptate	Terrestrial	Unidentified dead branches, China	This study
D. ganzhouensis	54–93 × 4.5–7	59–139 × 11.5–16.5	obclavate, pale brown to brown, 10–23-distoseptate	Terrestrial	Unidentified dead branches, China	This study
D. gasaensis	104–204 × 4–7.2	44–72 × 6–12	Obclavate or obpyriform, brown to pale brown, rostrate, 5–9-distoseptate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)
D. guanshanensis	15.4–44.7 × 5.2–8.3	96.5–255.3 × 12.3–16.5	Obclavate, pale brown to dark brown, (5–)11–38-distoseptate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)
D. hainanensis	70–130 × 5–8.5	44–117 × 9–18.5	Obclavate or obpyriform, rostrate, brown, verrucose, up to 22-distoseptate	Terrestrial	Unidentified decaying wood, China	Chen et al. (2024)
D. jinghongensis	54.6–94.6 × 3.6–4	56.4–127.3 × 7.3–10.9	Obclavate, rostrate, pale brown or brown, 7–17-distoseptate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)
D. lanceolatispora	120–190 × 4–8	31–90 × 9.5–15	Fusiform or lanceolate, rostrate, olivaceous to olivaceous brown, verrucous, with or without apical, hyaline appendages, 5–13-distoseptate	Freshwater	Unidentified submerged wood, China	Chen et al. (2024)
D. liupanshuiensis	70–340 × 3.5–7	55–90 × 6–11	Obpyriform, light brown below, hyaline towards apex, 8–10-distoseptate	Terrestrial	Dead culms of bamboo, China	Dissanayake et al. (2024)
D. longnanensis	77–171 × 4–8	54–87 × 8.2–14	Obclavate, olivaceous to yellowish-brown or brown, 4–8-septate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)
D. menghaiensis	45.7–82.9 × 3.4–5.1	35.7–48.6 × 7.2–10.9	Obclavate, pale brown to brown, 4–8-distoseptate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)
D. menglunensis	35–52.5 × 6.3–7.5	82.5–172.2 × 12.5–15	Obclavate, brown to dark brown, 14–33-distoseptate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)
D. nanpingensis	8.5–28 × 5–7	169–282 × 12–17.5	obclavate, sometimes with a swollen cell, reddish-brown and slightly paler towards the apex, sometimes with percurrent regeneration forming a secondary conidium from the conidial apex, 28–41-distoseptate	Terrestrial	Unidentified dead branches, China	This study

Species	Conidiophores (µm)	Conida		Habitat	Host/Locality	References
		Size (µm)	Morphology			
D. nanchangensis	18.2–76.4 × 5.5–8.0	149.1–292.7 × 10.9–17.8	Obclavate, brown to dark brown, sometimes with percurrent regeneration forming a secondary conidium from the conidial apex, apex sometimes with a germ tube or short germination hypha, (17–)21–43-distoseptate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)
D. narathiwatensis	27–155 × 3–6.5(–7)	12–38 × 4.5–8	Solitary or occasionally catenate, subcylindrical to obclavate to conical, olivaceous to brown, apex rounded, often the primary cells of conidia are narrower than the second ones which are often inflated, 1–7-distoseptate	Terrestrial	Dead petiole of *Eugeissona tristis*, Thailand	Karimi et al. (2024)
D. phragmiticola	50–145 × 5–8	34–155 × 10.5–17.5	Obclavate, rostrate, pale brown, grayish brown or mid-brown, paler towards the apex, 4–25-septate	Terrestrial	*Phragmites australis*, China	Hyde et al. (2023)
D. sichuanensis	15–25 × 4–6	80–145 × 6–17	Obclavate, elongated, yellowish brown to brown, hyaline at the apex, 12–20-distoseptate	Terrestrial	On dead twigs of unidentified host, China	Dissanayake et al. (2024)
D. suae	(21–)25–41(–53) × 4–5	(77–)81–101(–109) × 8–10	Obclavate to rostrate, bent at the second or third cell at the base, brown to dark brown, 3–12-euseptate, guttulate, verrucose	Freshwater	On submerged decaying branches, China	Shen et al. (2024)
D. subtropica	125–262 × 6–10.5	64–147 × 12–15	acropleurogenous, obclavate, rostrate, verrucose, pale brown to brown, 5–10-euseptate	Terrestrial	Unidentified dead branches, China	This study
D. xinpingensis	(97–)105–149(–175) × 4–5	(95–)107–139(–155) × (7–)8–9(–10)	Obclavate, brown, sometimes a second conidium proliferates at the top of the conidia, 8–12-euseptate	Freshwater	On submerged decaying branch, China	Shen et al. (2024)
D. yichunensis	17.9–52.7 × 5.3–6.8	113.7–272.7 × 12.2–16.9	Obclavate, pale brown to brown, (14–)22–35-distoseptate	Terrestrial	Unidentified dead branches, China	Hu et al. (2023)

Notes: All conidia are smooth and solitary, except where indicated.

a terrestrial habitat in China, while they are monoblastic in collections (MFLU 17–1653 and HKAS 125826) from terrestrial and freshwater habitats in Thailand and China, respectively. Additionally, the latter collection (HKAS 125826) shows longer conidiophores and conidia (Sun et al. 2020; Shen et al. 2024). Despite these morphological differences, the strains cluster together with high support in multi-locus phylogenetic analyses using ITS, LSU, *RPB2*, and *TEF1* (Shen et al. 2024). Given this phenomenon, we conclude that certain morphological characters, such as conidiogenesis, traditionally used for species or genus delimitation in sporidesmium-like genera may be insignificant in the current phylogenetic context. As a result, the development of molecular techniques and multi-locus phylogenetic analyses has become a powerful tool for delimiting *Distoseptispora* species in the modern fungal classification. Accordingly, based on previous studies, we proposed four new species and two known species in this study, distinguished by both morphological characteristic and multi-locus (ITS, LSU, *RPB2*, and *TEF1*) phylogenetic analyses.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

The concatenated LSU, ITS, *RPB2* and *TEF1* sequences

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Data type: fas

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